DESCENT WITH MODIFICATION: EVOLUTIONARY STUDY OF *COLIAS* IN THE TRADITION OF CHARLES REMINGTON

Ward B. Watt

Department of Biological Sciences, Stanford University, Stanford,
California 94305, USA and Rocky Mountain Biological Laboratory,
P.O. Box 519, Crested Butte, Colorado 81224, USA

ABSTRACT. Studies of pierid butterflies have for several decades been at the center of diverse advances in the understanding of microevolutionary processes. An important ingredient of this success is the intellectual model—a way of thinking—that Charles Remington has passed to his students and colleagues. This paper reviews the Remington paradigm as applied to research on the bioenergetics of natural populations of *Colias* butterflies, focusing on the impact of thermal and related environmental constraints on the allocation of metabolic resources, and the function and genetic variability of enzyme polymorphisms.

In 1951, Alexander Klots' landmark *Field Guide to the Eastern Butterflies* appeared. Therein, Klots remarked (p. 183) of *Colias*: "I know no other genus of butterflies that presents so many baffling, and stimulating, problems." Five years later, an aspiring high school student and lepidopterist, interested in natural polymorphism in *Colias* as the topic of a science fair project, wrote to Klots for information, and was referred to Charles Remington, who recently had published a major review (Remington 1954) on polymorphism in *Colias*. Because of Remington's legendary ability at encouraging imaginative study of Lepidoptera by amateurs and professionals, students and senior scholars alike, the rest is, as they say, history—for I was that student. Charles has been my friend and professional colleague for all of my adult life and more than half of his. It is a privilege to join his many other friends and intellectual descendants in marking his entry into his eighth decade with thanks and best wishes.

The Remington Scientific Tradition

If we want to understand Charles' success at fostering others' understanding as well as his own, we must go beyond well-deserved personal tributes to ask how he does it. Much of the work reported in this issue of the *Journal* embodies a clear, active tradition of scientific work in which Charles, and those who have learned from him, participate alike. This is a tradition in the best sense: doing things in the same grand style as one's forebears, but not necessarily doing the same things, or repeating particular deeds again and again.

First among the Remington themes is the importance of detailed knowledge of particular groups of organisms as well-understood "test beds" for study of general problems both in basic and applied science.

This is a very Whiteheadian (1938) viewpoint, emphasizing the human importance of both specific knowledge and general patterns of such knowledge. Charles' focus on the study of Lepidoptera, in context of both evolutionary studies and conservation biology, embodies this theme.

This in turn synergizes with Charles' long-standing commitment to the continuity of communication and intellectual effort between amateur and professional biologists, which we see in his co-founding of the Lepidopterists' Society. This positive sense of "scientific democracy" also has been important in his welcoming of generations of Yale University students into enthusiastic research collaboration.

Finally, we see qualities which revolve around Charles' enthusiasm for eclecticism of concepts and techniques in the broad study of evolution. He emphasizes the importance of large databases as sources of ideas: his students are encouraged to acquire much data, but to think about their meaning even as they come in, and his commitment to building the insect collections of Yale's Peabody Museum has been extremely successful. But Charles is no hyper-empiricist. He has long held that the prime importance of experimental or observational data in evolutionary biology is its use to test ideas, concepts, and theories. Yet, he insists, data must *drive* theory and not the reverse—if a theory clashes with new findings, so much the worse for that theory.

All the contributions to this *Journal* issue show many of the hallmarks of this tradition. My own work, using *Colias* as a "test bed" to study evolutionary mechanisms, has turned out to require all of them in order to address its central questions.

THE STUDY OF EVOLUTION, USING *COLIAS*, IN THE REMINGTON TRADITION

Klots' "baffling and stimulating problems" presented by *Colias* have their sources in the evolutionary biology of this apparently rapidly evolving group of butterflies. Naturally occurring variation can be a uniquely powerful probe with which to explore evolutionary problems like these. A wide variety of such natural variation in *Colias*—from dramatic color polymorphisms, to clinal variation in other aspects of color pattern or morphology, to polymorphisms in the enzymes that process energy in support of flight—all turns out to fit into a context of bioenergetics i.e., the study of energy flow through living systems. Complex patterns of such variation, within and among species, have indeed caused much of the confusion about *Colias*' classification to which Klots referred. However, we will see that, finally, careful study of the mechanisms and the molecular basis of this variation holds much promise for resolving the systematic confusion, as well as illuminating new general principles about the workings of evolution.

Cold Habitats, Hot Butterflies—Temperature Regulation and Flight in *Colias*

In the winter of 1962–1963, my first graduate student year, after four years as a Yale undergraduate with Charles' advice and tutelage, I was spending an afternoon with the *Colias* collections at Yale's Peabody Museum. After a while, I noticed that *Colias* from cold habitats tended to have dark (melanic) hindwing undersides. I already knew from much observation that *Colias* rest with their wings folded dorsally, and the thought occurred to me that this might be an adaptation for absorbing sunlight to warm up in cold climates. I mentioned this idea to Charles the next morning; he enthusiastically recommended I think of some way to run an initial biological test of the idea.

In the next few months, preparing for a (second) summer of field work at Rocky Mountain Biological Laboratory, in Colorado, it occurred to me that if *Colias* were using this hindwing underside patch as a thermoregulatory device, then when cold, they should turn to expose it perpendicular to sunlight, giving maximum warmup advantage. In late July 1963, then, I spent a morning at 12,200 feet on the south edge of the Mesa Seco, in Hinsdale County, Colorado, working with the alpine, heavily melanic *Colias meadii* Edwards. I put half a dozen fresh males, alive, each into a metal 35mm film can, then put them in the shade to cool down to ambient temperature in the cold alpine air. Releasing them one by one onto the sunlit tundra, I saw each one turn itself like a small sailboat, orienting perpendicular to the solar beam! Moreover, each was warm enough to fly off in only two or three minutes, though if they were tossed into the air directly out of their cans, they simply fell helplessly to the ground.

Here I had strong evidence that my idea was worth pursuing. Again, Charles was helpful. This sort of "environmental physiology," as it was then known, was beyond his personal experience, but he knew of an entomologist, Phillip Adams, who was experimenting with the implantation of minute thermistor devices non-destructively into flight muscles of butterflies, so as to monitor their thoracic temperatures during various activities. Adams kindly sent me plans and advice for building the needed field-portable equipment. Summer 1965 found me in northwestern Canada deploying this gear for the first time, to study the thermal biology of a series of *Colias* species which differed altitudinally in hindwing melanization: *C. alexandra christina* Edwards, *C. palaeno chippewa* Kirby, and *C. nastes streckeri* Grum-Grshmailo.

To make a long story short (see Watt 1968, 1969, Kingsolver 1983a, 1983b, Kingsolver & Watt 1984 for details), *Colias* of all species studied (the aforementioned, plus *C. a. alexandra* Edwards, *C. eurytheme*

Boisduval, *C. meadii* Edwards, and *C. philodice eriphyle* Edwards) require a high and narrow body temperature range for optimal flight—35–39°C—and do not fly voluntarily at all outside about 29–40°C. This in itself was remarkable: "environmental physiology" of the time led to the expectation that cold habitat populations would fly at lower body temperatures than warm habitat ones, but not so!

All *Colias* also proved to display the same behavioral responses to habitat temperature, orienting perpendicular to sunlight to warm up when cold, and orienting parallel to sunlight, or seeking shade, when overheated. Populations in different thermal habitats differ in their adaptive morphological mechanisms for achieving the common thermal optimum. The darkened hindwing underside in cold habitats, brighter in warm habitats, is indeed a temperature-regulating device, changing absorptivity for sunlight. Moreover, cold habitat populations have thicker thoracic "fur" (actually modified scales, of course) than do warm habitat populations, thus modifying their rates of heat loss via convective cooling. We now have been able to build a computer software model which, with a few measurements of a *Colias'* solar absorptivity, "fur" thickness, and the like, together with recorded data on air and ground temperatures, wind speed, and sunlight intensity in a habitat, can accurately predict when during the day that *Colias* will have the opportunity to fly (Kingsolver & Watt 1984). This is useful not only for understanding *Colias'* thermal habitat structure in its own right, but for carrying out other experiments in that context (see below, Watt 1992).

This analysis allows us to understand *Colias eurytheme*'s seasonal forms, "spring-fall" (=form "ariadne") versus "summer" (=form "amphidusa"), which Ae (1957) first showed to be due to photoperiodic sensitivity of *C. eurytheme* larvae. The light hindwing undersides of the summer form are less absorptive of sunlight, compared to the dark spring-fall phenotype (Watt 1969; this mechanism also cues the difference between light summer and dark spring-fall hindwing undersides of *C. p. philodice* Latreille (Watt, unpublished data)). Hoffmann (1973) showed that photoperiod alone controls this, reproducing the natural field alternation of forms quantitatively by reference to the response curve of hindwing color versus photoperiod under which larvae are reared in the lab. Recently, we have found (Jacobs & Watt 1994) that in *C. eurytheme*, photoperiod also cues deeper thoracic "fur" (giving more insulation against spring-fall convective cooling) in the short-day spring-fall forms than in the long-day summer ones. Both these differences contribute to greater flight time for each photoperiod form in the season in which it is produced and in which it flies (Jacobs & Watt 1994).

I think it is significant that the whole business of understanding *Colias*' thermoregulatory adaptations had its origin in the Yale Peabody Museum's extensive collection of Lepidoptera, which owes its existence to Charles Remington's tireless activity. This use of collection material to provoke active experimental study is a scientific value of collections which is all too often overlooked, but which will be of increasing importance in the future. I also note that all the original work was done with home-built, shoestring-budget equipment and direct behavioral observation, in a way perfectly accesible to serious amateur lepidopterists—indeed, even quite modern and sophisticated equipment for monitoring thermistor or thermocouple probes, usable for such work, remains relatively inexpensive and accessible.

The Sex-limited "Alba" Female Polymorphism

The white-female "alba" variant was really the first focus of my interest in *Colias,* leading me to write first to Klots and then to Remington when in high school. For a science fair project, I was trying to apply what was then a new, powerful biochemical separation technique—paper chromatography—to analyzing the effects of "alba" on *Colias*' yellow, orange, or white wing pigments (a class of compounds called pteridines, first isolated from the wings of European pierids).

The "alba" morph has a distinguished history in biological study, beginning with John Gerould's (1911) use of it to make the first demonstration of sex-limited Mendelian inheritance. William Hovanitz (trained as a physician, illustrating yet another position along the amateur/professional continuum, which has been so important to Lepidopteran evolutionary study) demonstrated in 1950 that many North American *Colias* species show parallel biogeographic gradients of "alba" frequency, with the coldest-habitat populations of any one species having the highest frequency of this morph, yet often with little correlation between species in this respect. When I first wrote to Remington, he had recently published his survey (1954) of the genetics of *Colias,* including a summary of what was known about "alba." This paper was a major stimulus to my own approach (and later, those of my students as well) to the "alba" problem.

Hovanitz (1948) also reported that "alba" had a propensity to fly in cooler parts of the day than its yellow or orange sisters. Unhappily, he was misled by deficiencies in his observational technique, as Leigh and Smith (1959) showed. In fact, having applied field thermal monitoring technique to address the meaning of *Colias*' underwing coloration, I then used the same technique to test Hovanitz' idea in another way (Watt 1973): the two morphs do not differ in their preference for flight temperatures, and moreover the color difference, to the extent it influ-

ences the insects' thermal balance, would force "alba" females to fly less in the cooler parts of the day. At this same time, my early interest in the chemistry of the wing pigments paid off: data obtained by paper chromatography and spectral analysis showed that "alba" is, biochemically, *not* a "simple oxidation" of colored to colorless pigments (cf. Ford 1953) but a redirection of expensive pigment precursors out of pteridine synthesis into other physiological uses during pupation. This might plausibly lead to physiological advantages for "alba" when pupae develop under cold stress.

Our next look at "alba" grew out of these results. Graham, Watt, and Gall (1980) found that, indeed, when *C. eurytheme* pupae develop at low temperature, "alba" are superior to their orange sisters in speed of maturation and in maturity of eggs when females eclose—but such effects diminish or vanish at warmer temperatures. In addition, we compared "alba"'s eclosion times, fat body sizes, and mating successes in the wild in two near-sympatric Colorado *Colias* which differ greatly in "alba" frequency: *C. a. alexandra*, which has "alba" frequency of 5% or less, and *C. scudderi* Reakirt, with "alba" frequency often in excess of 90%. Moreover, we made these comparisons in populations whose numbers, age distributions, and movement or dispersal patterns had already been studied (Watt et al. 1977) with the aid of yet another Remington doctoral student, Frances Chew (who was my first undergraduate research student).

We found that the physiological and developmental advantages for "alba" at low temperature were indeed seen in the wild, and "alba"'s advantage in emergence time was more pronounced in *C. scudderi*, which has a colder pupal habitat than *C. alexandra*. We also found an advantage to "alba" in mean fat body size for each adult age class, in both species. Following some data of Taylor (1972), another former Remington associate, we also examined the behavior of male *Colias* toward "alba" as compared to its yellow sisters (to do this, we developed a "tethering" technique for studying behavioral interactions between individual butterflies). We found that male *Colias* discriminate against "alba" in the initial visual phases of courtship, 5:1 in *C. alexandra* and 10:7 in *C. scudderi*; once the insects close in to centimeter distance (at which range their pheromones become effective; Silbergleid & Taylor 1978), the difference in male behavior toward the female morphs vanishes. The discrimination seems to be effective, in that "alba" have fewer matings than yellows in each age class (as we found by dissecting females and counting their spermatophores, in the manner of Burns 1968) in both species. Thus "alba"'s developmental advantages in the wild are opposed by behavioral disadvantages.

Claims of pierine distastefulness (e.g. Marsh and Rothschild 1974),

despite the observations of Wourms and Wasserman (1985), led some of our colleagues to suggest that mimicry of pierines by "alba" might be important to this polymorphism. With several students, I explored various implications of this idea: are *Colias* indeed more palatable to visual predators, notably birds, than pierines which are sympatric with them, so that mimicry might result?; and, what is the relation between "alba" frequency and sympatric pierine density? In pursuing the former idea, we developed techniques for studying butterfly palatability in a controlled fashion in natural populations (Ley & Watt 1989), and found that in a Colorado mountain ecosystem, there is no difference in palatability among *Pieris napi mcdunnoughi* Remington, *Pontia occidentalis* Reakirt, *C. a. alexandra*, and *C. scudderi*, and that none of these display the least sign of being distasteful to Canada jays (*Perisoreus canadensis* L.), though larger, heavier butterflies are eaten preferentially to these small pierids. Moreover, Watt, Kremen, and Carter (1989) found that "alba" frequency is negatively correlated, both in space and in time, with the density of these pierines. Thus we could definitively reject the 'mimicry' hypothesis for "alba." Indeed, we collected anecdotal observations to suggest that pierines constitute competitors with "alba," but not other *Colias* morphs, for reproductive space, based on visually mediated behavioral interactions.

The idea that "alba" and pierines are interference competitors may explain why male *Colias* are often selected to discriminate against "alba." This idea is now under further study, along with aspects of the biochemistry and physiology of "alba." The story is far from over, because what we know of the selection pressures should fix either one allele or the other in any given habitat, not maintain the polymorphism. But of course, we do see the polymorphism, in parallel, in many *Colias* species. Therefore there are still many important things to discover about this polymorphism, and we are still hard at it. Indeed, so are others: for example, Sappington & Taylor (1990) report the remarkable fact that "alba" and orange females of *C. eurytheme* preferentially mate with males having differing mixes of short-range courtship pheromones. How this might affect the mix of selection pressures maintaining the "alba" polymorphism is not yet clear.

Enzyme Variation, Energy Supply, and Fitness Measurement in *Colias*

Enzymes, the protein catalysts which control energy processing and other metabolic functions in all organisms, vary genetically in *Colias*' natural populations just as do visible morphology or color pattern. To visualize this variation, one uses the technique of gel electrophoresis, separating the proteins in an electric field on a gel support, followed

by enzyme-specific staining. In earlier decades, there was much debate about whether such enzyme variation, in a variety of organisms, is acted on directly by natural selection, or whether instead it is neutral in the course of evolution. Extreme positions on both sides of this debate are now widely thought to be misguided, with many workers arguing for a broad spectrum of situations, from intense selection on enzyme variation down to little or none (Endler 1986, Gillespie 1991, Watt 1994). In any event, in at least some cases such enzyme variants can be powerful probes of the organization of energy processing. We have found this to be so in *Colias*, exploring yet another aspect of our theme of bioenergetics.

We know by far the most about natural genetic variants in one enzyme, phosphoglucose isomerase or PGI, which occupies a place in the early part of the reaction pathway called glycolysis, the primary route for supplying carbohydrate-derived fuel to *Colias* adults' flight. Four alleles, hence ten genotypes, are frequent to common in the lowland species complex (*C. eurytheme, C. philodice eriphyle*). To summarize much detailed biochemical information, among these 10 genotypes there is 3–4-fold variation in the speed with which their PGI enzymes process energy-rich sugar phosphate compounds, and in general, the more effective a genotype's enzyme is in this task, the less stable it is in turn against thermal stress due to high body temperature (Watt 1983; this trade-off of kinetic power against thermal stability was predicted from general considerations of protein structure by Hochachka and Somero in 1973; ours is one of the best studied examples involving variation within a single species). From these biochemical data and consideration of the dependence of *Colias*' flight on its energy supply, in context of its thermal biology as already analyzed (above), we were able to predict that carriers of these enzyme genotypes should differ, in characteristic ways, in their ability to fly over the broad span of the day in the wild. These predictions have been amply supported by direct experimental field test in both *C. eurytheme* and *C. p. eriphyle* (Watt et al. 1983). In turn, since all *Colias* adult activities depend on flight in one way or another, we were able to predict that each of the components of Darwinian fitness—survivorship, male mating success, and female fecundity—should differ among these genotypes in ways predictable from their effects on flight. These predictions, too, have been tested in diverse populations and supported with great statistical significance (Watt 1977, 1983, 1992, Watt et al. 1985, 1986). In the most recent work, on female fecundity effects of the PGI genotypes, we used our computer model of *Colias*' thermal balance to separate out genotype-specific effects from the thermally determined variation in opportunity for all females to lay eggs each day (*Colias* lay single eggs
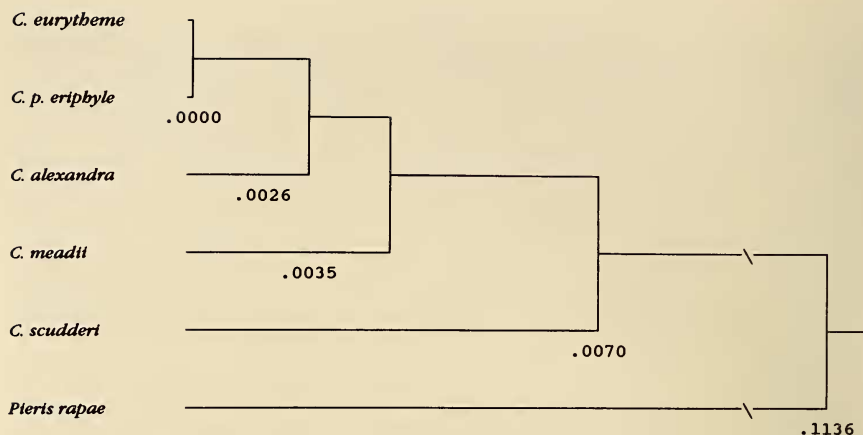
FIG. 1. Unweighted pair group, arithmetic mean distance tree of relationships among the given *Colias* taxa, with *P. rapae* as outgroup, based on initial mitochondrial DNA data of M. Ford, D. Pollock, W. Watt, and D. Rand (unpublished, summarized here by permission). A total of 757 base pairs is compared in this data set, drawn from portions of the 12s and 16s ribosomal RNA genes and the NADH dehydrogenase subunit 5 gene. Note that distances are very short within *Colias* (indeed, *C. eurytheme* and *C. p. eriphyle* show no differences as yet), reflecting a small number of differing sites in, and therefore specially conservative nature of, the sequences sampled. This is promising for the utility of more such sequence data in ordering relationships among species complexes. A cladistic analysis of the site variation patterns is also consistent with this tree.

and must fly between ovipositions). Thus, initially quite different parts of our research program are mutually reinforcing, showing with great clarity how natural selection can reach right down to the level of particular proteins.

A New Path for Re-exploring Old Problems—*Colias* and Molecular Systematics

The enzyme-level polymorphism just discussed occurs in parallel, just as does the "alba" polymorphism, in many different *Colias*, not just the North American lowland species complex. The opportunity to compare such evolutionary adaptations across a broad range of species seems attractive—but here problems arise, as the systematics of the genus is far from clear. Despite informative work in recent years by Ferris (1973) and others, often using new diagnostic characters such as those revealed by ultraviolet photography (Silbergleid & Taylor 1978), the placement of some *Colias* taxa into the proper species complexes is still controversial, and the relationships among species complexes are not at all clear. Indeed, the systematic reliability of some morphological characters that have been used to group taxa may be newly suspect. For example, some authors have grouped *C. nastes* Boisduval and *C. behri*

Edwards because of the very heavy melanization of their wings, which obscures the usual light pteridine pigment pattern. But this may well be the result of convergent evolution because of similarity in their bearers' thermal habitats, and not reflect close relationship.

We are beginning to deploy new molecular methods to resolve systematic problems in *Colias*, analyzing the insects' very DNA itself to generate data on the variation of DNA sequences at a variety of specific genes as a potentially powerful souce of systematic characters (which at the least will not likely be subject to the same kinds of biasing selection pressures as some morphological characters). Figure 1 displays a very preliminary tree of relationships among several North American *Colias* taxa, using *Pieris rapae* as an outgroup. These data, so far as they go, at least suggest that the North American legume-feeding *Colias* may share closer common ancestry than any of them do with the willow-feeding *C. scudderi*. Note also that in just over 750 base pairs of data thus far, no differences were seen between *C. eurytheme* and *C. p. eriphyle,* supporting an extremely close relationship between these "semi-species" (cf. Mayr 1963). Much more data-gathering and analysis will be needed to resolve the systematic difficulties of *Colias*, but these techniques do hold great promise for such resolution.

Environmental Applicability of *Colias* Research

Not only does the above work have implications for fundamental Darwinian biology at large, and for specific understanding of these pierid butterflies, but much of it appears to be applicable to environmental problems as well. I give two examples.

First, one of the great problems confronting the management of endangered species of small animals is that because of their fragile condition, one cannot study their population genetics—thus leaving us ignorant as to what genetical difficulties their population size restrictions may be running them into. For example, this situation plagues attempts to conserve *Boloria acrocnema* Gall and Sperling in central Colorado (A. Seidl, personal communication). A number of montane or alpine *Colias* occur in some parts of their ranges as isolated local populations, though none of the taxa as a whole are yet near danger of extinction in North America. Genetical study of such isolated populations might be informative, in context of our population genetic insights in other *Colias*, as models for genetical constraints that may be occurring in local populations of already endangered Lepidoptera or other creatures.

Second, our finding of support, in the *Colias* PGI polymorphism, for Hochachka and Somero's tradeoff of enzyme kinetic function against thermal stability raises the possibility of unanticipated genetic consequences of global warming, should this occur. The most thermally stable

PGI genotypes, because of their disadvantages in energy processing rate at normal temperatures, have far lower male mating success or female fecundity than do other genotypes in the system—yet these are the genotypes which would best survive the increase in acute overheating which even moderate global warming would quickly impose on *Colias*. The initial result of this "selection for poor reproduction" would be a dramatic drop in population sizes, probably throughout the range of whole species. How fast natural selection could work on the insects' thermoregulatory adaptations to recover population size would then be an open question. In any case, this would raise the probability of their extinction, especially for cold-habitat montane and alpine species which may already occur in separated local populations. We are beginning to explore this grim possibility quite actively, with respect to *Colias* and other butterflies, and also to see how far such effects might run through the animal and plant world at large.

SUMMARY AND CODA

Colias butterflies now appear as a "test bed" system with great potential for ever-diversifying future work in fundamental and applied biology alike. Our results re-emphasize the importance of collections in sparking new scientific ideas, the importance of applying diverse analytic techniques to complex scientific problems, and the crucial interaction of extended databases with a sufficient variety of alternative explanations for natural phenomena. Again, I would emphasize that many of these kinds of study are accessible to the serious amateur lepidopterist just as much as to the professional. All these things are very much in the Remington tradition.

Let no one suppose, however, that in inspiring those of us who represent the Remington tradition in this *Journal* issue, Charles has completed his scientific and educational tasks. No indeed—he is still actively innovating, in path-breaking ways, in his own tradition, as shown by his recent brilliant work with Gary Bernard (see Bernard & Remington 1991) on the visual physiology of *Lycaena* coppers in relation to their mate choice mechanisms and other aspects of their behavioral biology. Moreover, he continues to inspire enthusiasm in new students and colleagues, by daily interactions at Yale and elsewhere, as well as by example not only to us, his intellectual offspring, but to our students in turn, Charles' intellectual grand- and greatgrandchildren. Thus, by intellectual "descent with modification," there will be even more young scientists forthcoming who owe thanks to this versatile, energetic scholar-teacher. Who knows what he will do next? For certain, it's worthwhile to watch and find out. . . .

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